Electronic Supplementary Material (ESI) for Analytical Methods.

Supplemental information for:

A 3D-Printed, Multi-Modal Microfluidic Device for Measuring Nitric Oxide and ATP Release from Flowing Red Blood Cells

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Figure S1. CAD designs of the double mixing T multi-modal device.
Figure S2. Characterization of mixing in the single mixing T device.
Figure S3. Effect of the luciferin/luciferase flow rate on peak height (ATP detection).
Figure S4. Interference plots for NO and ATP detection.
Figure S5. Standard addition plots for quantitating ATP in normoxic and hypoxic RBC solutions.

SI also contains .STL file of the double T device used in these studies.

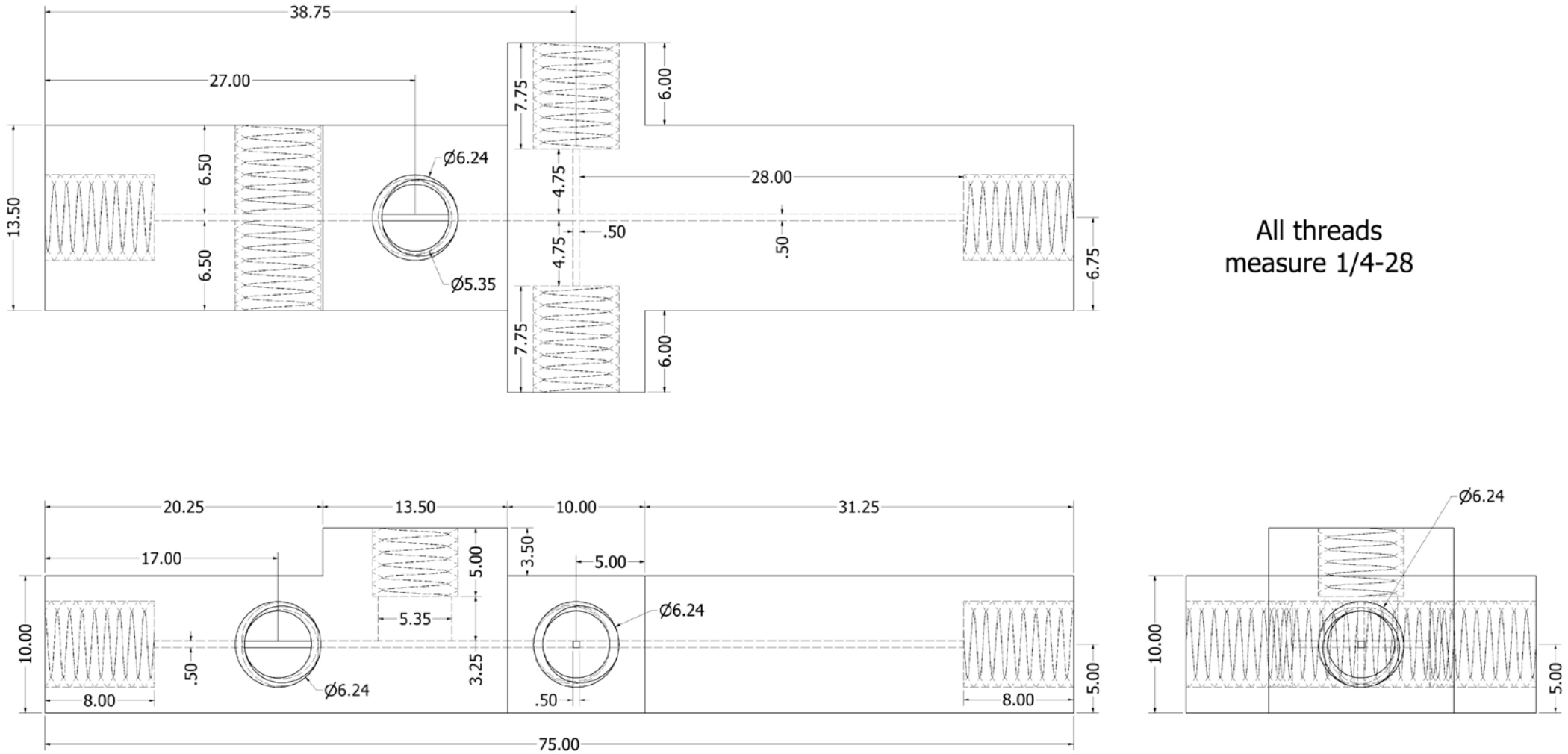


Figure S1: CAD designs of the double mixing T multi-modal device. Dimensions are in mm.

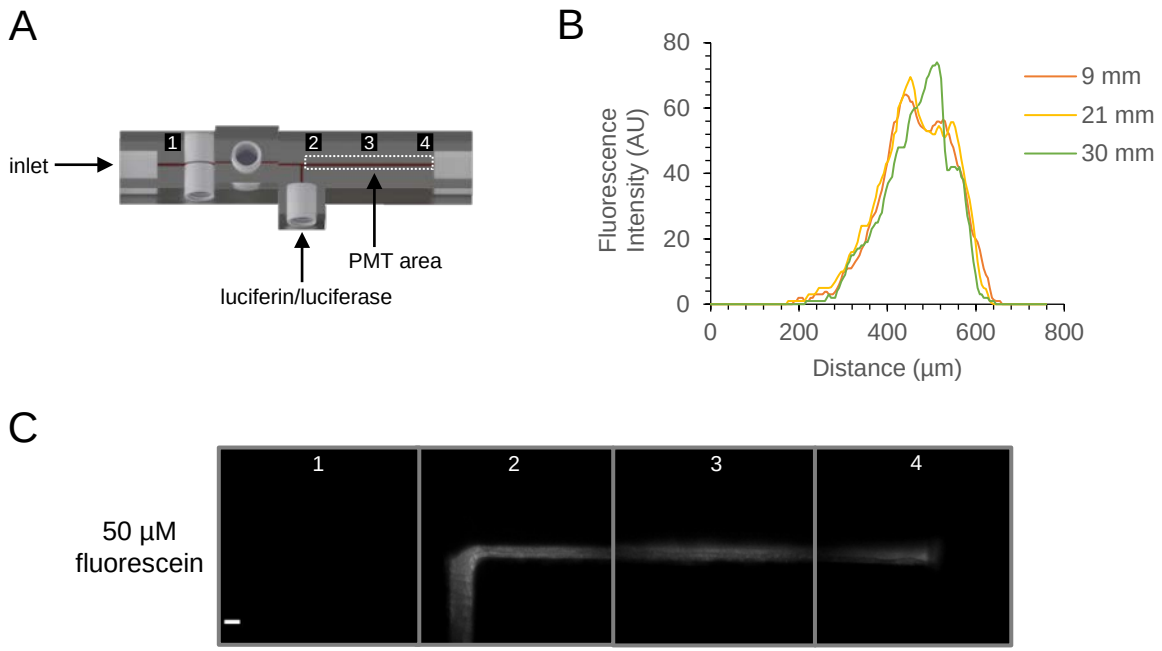


Figure S2: Characterization of mixing in the single mixing T device. A) CAD rendering of the device that illustrates the imaging points before and across the mixing channel. B) Graph showing the fluorescence intensity (line scan) across the mixing channel as a function of the distance downstream from the single T intersection. C) Micrographs of fluorescein at discrete imaging points before and across the mixing channel (scale bar = 100 µm).

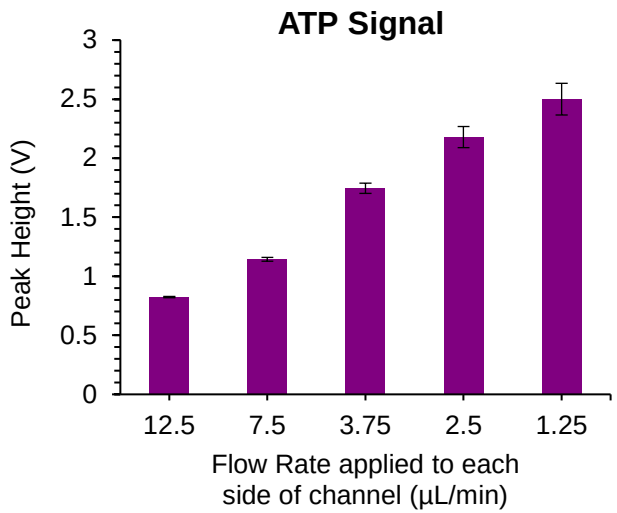
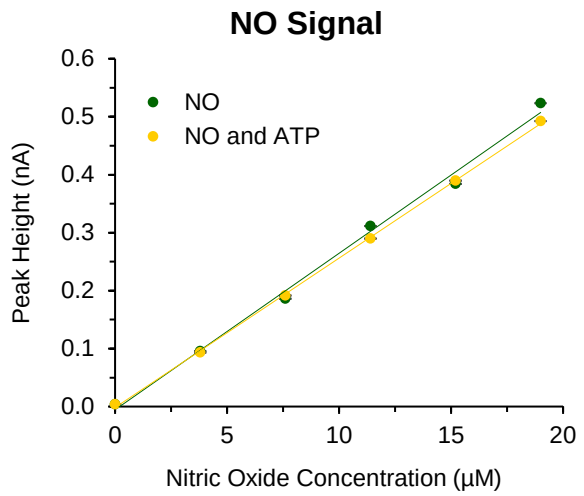


Figure S3: Effect of the luciferin/luciferase flow rate on peak height (ATP detection). The average response for 3 injections of a 2.5 µM ATP solution are shown for each flow rate (the reported flow rates were applied to each side channel).

A

Sample	NO	ATP	NO and ATP
1	0 µM NO	0 µM ATP	0 µM NO + 0 µM ATP
2	3.8 µM NO	0.5 µM ATP	3.8 µM NO + 0.5 µM ATP
3	7.6 µM NO	1.0 µM ATP	7.6 µM NO + 1.0 µM ATP
4	11.4 µM NO	1.5 µM ATP	11.4 µM NO + 1.5 µM ATP
5	15.2 µM NO	2.0 µM ATP	15.2 µM NO + 2.0 µM ATP
6	19 µM NO	2.5 µM ATP	19 µM NO + 2.5 µM ATP

B



C

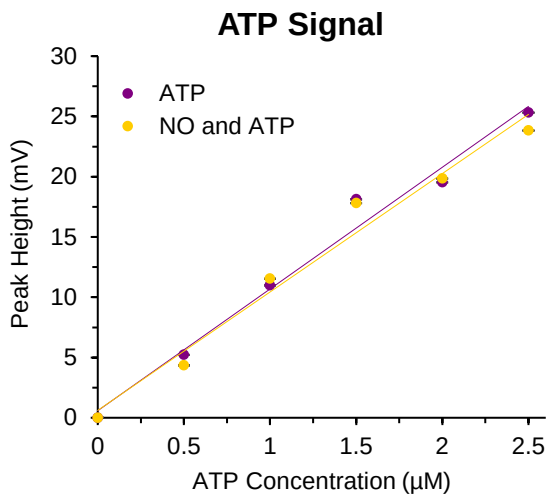


Figure S4: Interference plots for NO and ATP detection. A) Samples were made in PBS according to the table. B) Calibration curve for NO alone and for NO and ATP. C) Calibration curve for ATP alone and for NO and ATP.

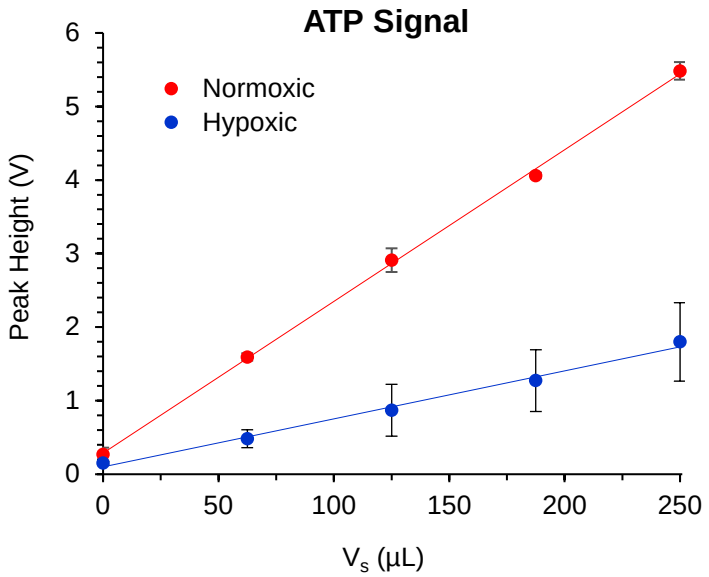


Figure S5: Standard addition plots for quantitating ATP in normoxic and hypoxic RBC solutions. Deoxygenated hemoglobin absorbs light at the maximum emission wavelengths of the luciferin/luciferase reaction, attenuating the signal and necessitating separate curves for normoxic and hypoxic measurements.